Registration of 'Clearwater' Low-Phytate Hulless Spring Barley

P. Bregitzer,* V. Raboy, D. E. Obert, J. Windes, and J. C. Whitmore

ABSTRACT

'Clearwater' (Reg. No. CV-335; PI 647080) is a spring two-rowed barley (*Hordeum vulgare* L.) developed by the USDA-ARS and the Idaho Agricultural Experiment Station. Clearwater was selected and released on the basis of competitive agronomic performance in combination with low-phytate (LP), hulless grain. Clearwater derives from an $BC_1F_{3:4}$ selection from the cross 'Baronesse'*2/Pmut640//HB317. It was tested under the experimental designation 01ID435H. The parent Pmut640 is a sodium-azide generated mutant induced in the two-rowed malting cultivar Harrington. The mutation conferring reduced phytate in Pmut640 is believed to be allelic to *lpa2-1*, which results in a reduction of phytate (*myo*-inositol 1,2,3,4,5,6-hexa*kis*phosphate) of approximately 40 to 50% and an increase in inorganic P of greater than 400%. Studies have shown that these changes are associated with increased feed quality and reduced environmental impacts when fed to non-ruminant animals, based on increased P digestibility and reduced fecal P content.

'Clearwater' (Reg. No. CV-335; PI 647080) is a hulless, spring, two-rowed barley (*Hordeum vulgare* L.) tested under the experimental designation 01ID435H that was developed and released by the USDA-ARS and the Idaho Agricultural Experiment Station. Clearwater was developed primarily to address the anticipated demand for hulless feed barley cultivars with low phytate (LP) and high available P concentrations in the grain. These characteristics are associated with increased feed quality and reduced environmental impacts when fed to nonruminant animals, based on increased P digestibility and reduced fecal P content. Clearwater is the first low-phytate, hulless barley to be released as a cultivar. Clearwater was also selected on the basis of grain yields that are competitive with other specialty use (such as high β-glucan) barley cultivars, high test weight, and resistance to lodging. Clearwater is expected to be widely adapted to irrigated and rainfed conditions characteristic of the northern regions of the intermountain western United States (Colorado, Idaho, Montana, Washington, and Oregon). It is not believed to possess the necessary levels of resistance to foliar leaf diseases that are critical for commercial success in the upper Midwest.

Clearwater has the pedigree 'Baronesse'*2/Pmut640//HB317. Baronesse is a two-rowed feed barley that is well-adapted to irrigated and rainfed production areas in the western United States. It was developed by Nordsaat in Germany and is marketed in the United States by WestBred, LLC (Bozeman, MT). Its pedigree is 'Mentor'/'Minerva'//Vada (CIho 10086) mutant/4/'Carlsberg' (PI 163337)/'Union' (PI 28042)//'Opavsky' (PI 268172)/'Salle'/3/'Ricardo' (PI 328933)/5/'Oriol' (PI 346404)/6153P40. HB317 is a two-rowed hulless barley breeding line developed by the University of Saskatchewan, Saskatoon, Canada, and is a sister line (B. Rossnagel, personal communication, 2007) of 'CDC Dawn' (registered in Canada under CFIA variety reg no. 4146, May 1995). The parent Pmut640 is a sodium-azide generated mutant induced in the two-rowed malting cultivar Harrington (Harvey and Rossnagel, 1984). This mutation confers a significant reduction in grain phytate (*myo*-inositol 1,2,3,4,5,6-hexa*kis*phosphate) and a significant increase in inorganic phosphate. The mutation has been mapped to chromosome 7H (G. Hu, personal communication, 2007) and is therefore believed to be allelic to *lpa2-1* (Larson et al., 1998). The effects of *lpa2-1* on agronomic and quality characteristics have been evaluated previously (Bregitzer and Raboy, 2006a,b).

Methods

Clearwater was developed using a modified pedigree breeding procedure. The initial cross was made and advanced to the BC_1F_3 generation in the greenhouse. The initial selection for LP homozygotes was based on the single kernel evaluations of BC_1F_3 seed harvested from a small (98 plants) BC_1F_2 population. Five kernels from each plant were weighed, crushed, extracted overnight in

P. Bregitzer, V. Raboy, and D.E. Obert, USDA-ARS, National Small Grains Germplasm Research Facility, 1691 S. 2700 W., Aberdeen, ID 83210; J. Windes, Univ. of Idaho Research and Extension Center, 1693 S. 2700 W, Aberdeen, ID 83210; J.C. Whitmore, Univ. of Idaho Research and Extension Center, Tetonia, ID 83436. Received 13 July 2007. *Corresponding author (phil.bregitzer@ars.usda.gov).

Abbreviations: LP, low phytate; P_i, inorganic phosphorus; WRDSBN, Western Regional Dryland Spring Barley Nurseries; WRSBN, Western Regional Spring Barley Nurseries.

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677 S. Segoe Rd., Madison, WI 53711 USA

10 µL 0.4 M HCl per mg of kernel tissue, and 10 µL of the extract was assayed for inorganic phosphorus (P_i) using a modification of Chen's assay (Chen et al., 1956). Extracts from each kernel were placed in individual wells of 96-well microtiter plates along with 90 µL of deionized H_2O, 100 µL of "Chen's reagent" (1 volume 3 M H_2SO_4, 1 volume 20 mM ammonium molybdate, 1 volume 0.57 M ascorbic acid, and 2 volumes deionized H_2O), incubated for 2 to 4 h, and scored against various standards (0, 155, 465, 930, 1395, 1860, and 2325 ng P supplied as K_2HPO_4) using a microtiter plate reader equipped with an 810 nm filter. Kernels for which assayed extracts showed 930 ng P or more were considered to be LP. Plants from which all five kernels were LP were classed as putatively homozygous for the LP mutation and advanced for testing.

Ten BC_1F_2 plants homozygous for LP and hulless kernels were identified and advanced as $BC_1F_{2:3}$ families sown at a low population density. Each population was sown in four-row plots, and each row was sown on approximately 36 cm centers at a rate 1.5 g seed m^{-1} or less, in the field under irrigation in 2000 at Aberdeen, ID. Selections were made on the basis of overall agronomic appearance, with emphasis on favorable spike characteristics, especially with respect to kernel plumpness. A total of 49 plants from 8 of the 10 $BC_1F_{2:3}$ families were selected, reevaluated to confirm homozygosity for the LP mutation, and advanced for further evaluation as $BC_1F_{3:4}$ rows in 2001. Clearwater was derived from row no. 435, which was given the experimental line designation 01ID435H.

Clearwater was entered in preliminary yield trials in 2002 at a single, irrigated location, Aberdeen, ID, and was selected for further testing based on plant characteristics (height, maturity, and resistance to lodging) and productivity characteristics (grain yield, test weight, kernel plumpness). The experimental design was a randomized complete block with two replicates. From 2003 through 2006, testing was expanded to include additional sites in Idaho that varied with respect to elevation, sowing and harvest dates, and water availability (Table 1). These sites provide a wide range of conditions, and the highest average nursery yield is typically double that of the lowest nursery average yield. The primary sources of stress are typically abiotic (temperatures >30°C and/or limited moisture). The experimental design at each location was a randomized complete block with either three or four replicates. Soil fertility was managed according to soil test results and recommendations for yield goals appropriate for the site based on site characteristics, including considerations of production histories, anticipated water availability, and avoidance of excessive lodging. All plots were sown with small-plot drills equipped with double-disc openers. Each plot consisted of seven rows on 17.8-cm centers, and—depending on the location and year tested—varied from approximately 2.4 to approximately 3 m in length (length was consistent within any given trial). Plot arrangement was such that the experiments consisted of ranges of 7 to 20 side-by-side plots. Each range was separated by an alley approximately 1 m in width. Harvest was accomplished by small-plot combines.

Clearwater was tested also in the 2006 Western Regional Spring Barley Nursery and in the 2006 Western Regional Dryland Spring Barley Nursery (for details, see Erickson, 2007).

Data were collected during the testing of Clearwater included days to heading (date of head emergence from the boot for 50% of plants; visual estimate), plant height, percentage of lodged plants (visual estimate), grain yield, test weight, and percentage plump kernels (defined as the percentage of kernels retained on a sieve with 19.1- × 2.38-mm rectangular openings). Grain samples were also analyzed for P_i, total P, β-glucan, and protein. Total P, P_i, phytate, and β-glucan content determinations were made as described previously (Bregitzer and Raboy, 2006a; Dorsch et al., 2003). The total nitrogen content in seed samples was measured by AACC Method 46-30 (American Association of Cereal Chemists, 2000), using a protein analyzer (Model FT528, Leco Corp., St. Joseph, MI). The protein content was calculated based on total nitrogen with a conversion factor of 5.75. Moisture content was determined according to an AACC method (44-11) (American Association of Cereal Chemists, 2000). The moisture content was used to convert protein content into a dry matter basis.

Data were analyzed by SAS v. 8.0 Proc GLM (SAS Institute Inc., Cary, NC), utilizing the PROC GLM procedure, using various statistical models. Typical models included cultivar, locations, and years as sources of variance, with all sources of variance considered random except for cultivar.

Clearwater was observed to be uniform in appearance and for the LP characteristic from the F_4 through F_8 generations. Breeders seed was produced by bulking approximately 300 $F_{7:8}$ rows that were uniform and indistinguishable from each other in appearance. The LP phenotype was confirmed by selecting 40 kernels at random from the Breeders seed bulk and conducting single-kernel determinations of P_i as described above.

Table 1. Idaho locations used for the evaluation of 01ID435H ('Clearwater') barley.

Location	Elevation above sea level	Typical growing season	Irrigation	Year(s) used as a test site
	m			
Aberdeen	1338	early Apr. to early Aug.	sprinkler	2003–2006
Ashton	1698	mid-May to mid-Aug.	rainfed	2005–2006
Craigmont	1094	early Apr. to early-Aug.	rainfed	2003–2004
Fenn	1094	early Apr. to early-Aug.	rainfed	2006
Filer	1064	mid-Mar. to mid-July	furrow	2003–2006
Idaho Falls	1422	early Apr. to early Aug.	sprinkler	2006
Potlatch	790	early Apr. to early Aug.	rainfed	2003–2006
Rupert	1277	early Apr. to early Aug.	sprinkler	2006
Soda Springs	1763	mid-May to mid-Aug.	rainfed	2004–2006
Tammany	430	mid-Mar. to mid-July	rainfed	2004–2005
Tetonia	1794	mid-May to mid-Aug.	rainfed	2005–2006

Characteristics

Clearwater is a hulless, low-phytate, spring, two-rowed barley. It has an erect juvenile growth habit. Adult plants are medium-tall. Stems have six nodes, a closed collar, moderate head exsertion, and a strap-shaped, semi-lax spike that nods at maturity. Lemma awns are long and rough. Glume awns are rough, glume hairs are banded, and rachilla hairs are long. Rachis edges are covered with hairs. Hulls have prominent, barbless,

lateral veins, and a depression tending toward a crease at the base. Aleurone is white.

The mutation conferring the LP characteristic in Clearwater may be allelic to *lpa2-1*, as evidenced by phenotypic linkage analyses (V. Raboy, G. Hu, unpublished data, 2007). This mutation has been extensively evaluated in the Harrington background (Bregitzer and Raboy, 2006a; Dorsch et al., 2003) and shown to confer significant reductions in grain phytate and significant increases in grain P_i, but it does not significantly change total P levels. Comparisons to Baronesse, which is a parent of Clearwater and is widely grown in the Intermountain West for feed, indicate that the LP mutation in Clearwater had similar effects (Table 2). Clearwater also showed significant increases over Baronesse for grain protein and β-glucan contents in these tests (Table 2).

Replicated tests for agronomic characteristics have been conducted in multiple irrigated and rainfed environments in Idaho, and in the 2006 Western Regional Spring Barley Nurseries (WRSBN) and the 2006 Western Regional Dryland Spring Barley Nurseries (WRDSBN). The data indicate that Clearwater has favorable characteristics for maturity, plant height, and resistance to lodging. Tests conducted at six Idaho locations (four rainfed and two irrigated) in 2005 and 2006 show Clearwater to be competitive with other hulless barley cultivars for yield, test weight, and percentage plump kernels, but it yielded significantly less than the hulled feed cultivar Baronesse (Table 3; Erickson, 2007). Clearwater also yielded less than the hulless cultivar CDC McGwire in 22 location-years of testing at various Idaho locations (Table 4). This difference was not significant under rainfed conditions, indicating that Clearwater is not especially sensitive to suboptimal moisture availability. Comparisons of Clearwater to the widely adapted feed cultivars Baronesse and Steptoe also showed relatively stable yield performance. The grain yield of Clearwater from the tests summarized in Table 3 was 73% of Baronesse in both irrigated and rainfed environments (data from individual environments not shown). The grain yield of Clearwater in the WRSBN and WRDSBN was 77 and 79%, respectively, of the yields of both Baronesse and Steptoe. Despite these indications of wide adaptability, Clearwater may not be a good choice for conditions of extreme moisture stress, as the *lpa2-1* mutation was associated with an intolerance for the unusually dry and hot conditions encountered during the agronomic tests conducted in 2002 and 2003 (Bregitzer and Raboy, 2006b). In these trials, the grain yield of the check cultivar, Harrington, under rainfed conditions was only 20% of that under irrigated conditions, whereas the data in the current report come from trials where the rainfed mean nursery yields ranged from approximately 65 to 80% of the irrigated mean nursery yields.

Table 2. Characteristics of 'Clearwater' and 'Barnoesse' barley produced in irrigated and rainfed Idaho environments in 2005 and 2006 (16 location-years).

	Inorganic P	Total P	Inorganic P/total P	Phytate P†	Protein	β-glucan
	mg g^{-1}	mg g^{-1}		mg g^{-1}	g kg^{-1}	g kg^{-1}
Clearwater	1.00***	3.13	0.32***	1.44***	129.1***	43.8**
Baronesse	0.22	3.11	0.07	2.67	109.5	41.0

**Significantly different from Baronesse values at $p < 0.01$.

***Significantly different from Baronesse values at $p < 0.001$.

†Data from 2005 and 2006, Aberdeen, only.

Clearwater has been tested primarily under conditions that are not conducive to disease, and little is known about its resistance to common barley diseases. Clearwater is not expected to be well adapted to environments characterized by high disease pressure.

Availability

Small quantities of seed to be used for research or breeding purposes can be requested from the corresponding author. A sample of Clearwater has been deposited with the USDA, National Plant Germplasm Center, and requests for small quantities of seed can be made via their Germplasm Resources Information Network website (http://www.ars-grin.gov/npgs).

Foundation seed of Clearwater will be maintained by the Idaho Agricultural Experiment Station, Foundation Seed Program. Requests for seed should be directed to the Coordinator, Foundation Seed Program, College of Agriculture, Kimberly

Table 3. Agronomic performance of 'Clearwater' and selected check cultivars from two irrigated and four rainfed Idaho locations, 2005 and 2006.

	Grain characteristics†	Heading	Height	Lodging	Yield	Test weight	Plump kernels
		d from 1 Jan.	cm	%	kg ha^{-1}	kg m^{-3}	%
Baronesse	hulled	180	29	6	5020	691	84
Bear	hulless	181	30	9	4166	744	40
Clearwater	hulless LP	179	31	15	3677	755	56
CDCAlamo	hulless HBG	182	29	12	3338	763	63
Waxbar	hulless waxy	185	31	40	2763	731	35
Azhul	hulless HBG	175	23	12	1876	730	74
	LSD $p = 0.05$	2.4	1.8	8.1	538	23	7.6

†LP, low phytate; HBG, high β-glucan.

Table 4. Agronomic performance of 'Clearwater' and 'CDC McGwire' in rainfed (12 location-years) and irrigated Idaho environments (10 location-years), 2003–2006.

	Irrigation	Heading	Height	Lodging	Yield	Test wt.	Plump kernels
		d from Jan. 1	cm	%	kg ha^{-1}	kg m^{-3}	%
CDC McGwire	rainfed	186	76	10	3709	770	32
Clearwater	rainfed	184**	74	18	3548	745**	46**
CDC McGwire	irrigated	175	84	30	5698	797	53
Clearwater	irrigated	174*	81	35	4891**	759***	69***
CDC McGwire	all locations	181	79	23	4622	782	41
Clearwater	all locations	179***	79	29	4139**	752***	57***

*Significantly different from CDC McGwire values within an irrigation regimen at $p < 0.05$.

**Significantly different from CDC McGwire values within an irrigation regimen at $p < 0.01$.

***Significantly different from CDC McGwire values within an irrigation regimen at $p < 0.001$.

Research and Extension Center, 3793 N 3600 E, Kimberly, ID 83341. It is requested that appropriate recognition of source be given when this cultivar contributes to research or development of new breeding lines or cultivars.

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